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Assessment of drinking water quality using ICP-MS and microbiological methods in the Bholakpur area, Hyderabad, India

Rasheed M. Abdul · Lakshmi Mutnuri · Patil J. Dattatreya · Dayal A. Mohan

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Abstract A total of 16 people died and over 500 people were hospitalized due to diarrhoeal illness in the Bholakpur area of Hyderabad, India on 6th May 2009. A study was conducted with immediate effect to evaluate the quality of municipal tap water of the Bholakpur locality. The study consists of the determination of physico-chemical properties, trace metals, heavy metals, rare earth elements and microbiological quality of drinking water. The data showed the variation of the investigated parameters in samples as follows: pH 7.14 to 8.72, EC 455 to 769 μS/cm, TDS 303.51 to 515.23 ppm and DO 1.01 to 6.83 mg/L which are within WHO guidelines for drinking water quality. The water samples were analyzed for 27 elements (Li, Be, B, Na, Mg, Al, Si, K, Ca, V, Cr, Mn, Fe, Ni, Co, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Sb, Ba and Pb) using inductively coupled plasma-mass spectrometry (ICP-MS). The concentrations of Fe (0.12 to 1.13 mg/L), Pb (0.01 to 0.07 mg/L), Cu (0.01 to 0.19 mg/L), Ni (0.01 to 0.15 mg/L), Al (0.16 to 0.49 mg/L), and Na (38.36 to 68.69 mg/L) were obtained, which exceed the permissible limits of the World Health Organization (WHO) for drinking water quality guidelines. The remaining elements were within the permissible limits. The microbiological quality of water was tested using standard plate count, membrane filtration technique, thermotolerant coliform (TTC), and most probable number (MPN) methods. The total heterotrophic bacteria ranged from 1.0×10^5 to 18×10^7 cfu/ml. Total viable bacteria in all the water samples were found to be too numerable to count and total number of coliform bacteria in all water samples were found to be of order of 1,100 to >2,400 MPN index/100 ml. TTC tested positive for coliform bacteria at 44.2°C. All the water samples of the study area exceeded the permissible counts of WHO and that (zero and minimal counts) of the control site (National Geophysical Research Institute) water samples. Excessively high colony numbers indicate that the water is highly contaminated with microorganisms and is hazardous for drinking purposes. Bacteriological pollution of drinking water supplies caused diarrhoeal illness in Bholakpur, which is due to the infiltration of contaminated water (sewage) through cross connection, leakage points, and back siphoning.

Keywords Coliforms · ICP-MS · MPN · Trace elements · Water quality

R. M. Abdul (✉) · L. Mutnuri · P. J. Dattatreya · D. A. Mohan
Microbiology Laboratory, National Geophysical Research Institute, Council for Scientific and Industrial Research (CSIR), Room no. 180, Second floor, Geochemistry Building, Hyderabad, Andhra Pradesh, India, 500007
e-mail: rasheedmicro@rediffmail.com

Introduction

Water is basic to life and health; over 1 billion people worldwide have no access to safe drinking water (Peeler et al. 2006). Drinking water is, worldwide, the most significant single source of gastro-enteric diseases and one of the major causes of morbidity and mortality worldwide, mainly due to the faecally contaminated raw water, failures in the water treatment process or recontamination of treated drinking water (WHO 2003, 2004). The World Health Organization has estimated that diarrhoea will kill annually 2.2 million people worldwide (WHO 2006). Diarrhoeal illness remains a major killer in children and it is estimated that 80% of all illnesses in developing countries is related to water and sanitation; and that 15% of all child deaths under the age of 5 years in developing countries result from diarrhoeal diseases (WHO/UNICEF 2000, 2004; Thompson and Khan 2003). Lang et al. (2001) have estimated that 35% of the total reported gastroenteritis was water-related.

Ideally, drinking water should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution. Detection of faecal indicator bacteria in drinking water provides a very sensitive method of quality assessment (WHO 1993). Total coliform bacteria (excluding *E. coli*) occur in both sewage and natural waters. Some of these bacteria are excreted in the faeces of humans and animals, but many coliforms are heterotrophic and able to multiply in water and soil environments. Total coliforms can also survive and grow in water distribution systems, particularly in the presence of biofilms (WHO 2004). The use of bacteria as water quality indicators can be viewed in two ways: first, the presence of such bacteria can be taken as an indication of faecal contamination of water and thus as a signal to determine why such contamination is present, how serious it is and what steps can be taken to eliminate it; second, their presence can be taken as an indication of the potential danger of health risks that faecal contamination poses (Papini et al. 2005; Ryu et al. 2005; McQuaig et al. 2006). Therefore, monitoring the quality of water is one of the essential issues of drinking water management (Trevett et al. 2004; Dieter and Muckter 2007). The microbiological safety of water supplies is at present assured by monitoring for the absence of the total coliform bacteria. The total coliform group is a large collection of different kinds of bacteria. The faecal coliform group is a sub-group of total coliform and has fewer kinds of bacteria. *E. coli* is a subgroup of faecal coliform. To reliably assess the level of faecal contamination of water and thus the possibility for occurrence of enteropathogenic microbes, number of indicators have been proposed, amongst which *E. coli* is considered to be superior as an indicator of faecal contamination and hygienic quality of drinking water (Edberg et al. 2000; Ashbolt et al. 2001). *E. coli* is a type of faecal coliform bacteria commonly found in the intestines of animals and humans (Edrington et al. 2006; Mallin et al. 2007). Waterborne transmission of pathogenic *E. coli* has been well documented for recreational and contaminated drinking waters. A well-publicized waterborne outbreak of illness caused by *E. coli* O157:H7 (and *Campylobacter jejuni*) occurred in the farming community of Walkerton in Ontario, Canada (WHO 2004). Although most strains are harmless, some strains produce a powerful toxin and can cause severe illnesses. Infection often causes severe bloody diarrhoea and abdominal cramps; sometimes the infection causes non-bloody diarrhoea (Derlet and Carlson 2006; Blatchley et al. 2007). The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination.

Heavy metals are metals with high molecular weights that are of concern because they are generally toxic to animal life and human health if naturally occurring concentrations are exceeded. Heavy metal toxicities are relatively uncommon. However, failure to recognize and treat heavy metal toxicities can result in significant morbidity and mortality. Dehydration is common. Encephalopathy is a leading cause of mortality in patients with both acute and chronic heavy metal toxicity. At lower doses, copper ions can cause symptoms typical of food poisoning (headache, nausea, vomiting and diarrhoea). Among outbreaks with quantitative data, the lowest copper concentrations associated with effects were about 4 mg/l or higher reported lower effect levels in children (Knobeloch 1994; Stenhammar 1999). In recent times, interest in studies pertaining to heavy metal contamination in aquatic environment has increased in view of their harmful effects on human health. Antimony, arsenic, barium, beryllium, boron, cadmium, cobalt, lead, mercury, molybdenum, selenium, tin, uranium and vanadium are all implicated although the acceptable intakes and physiological effects are known for all of these elements. Consuming waters with lead and cadmium can cause serious health hazards. Elements such as lead and cadmium in groundwater have recently received special attention as indicators of pollution as well as from the medical point of view (Trieff 1980). Heavy metal toxicity has emerged as a significant occupational hazard associated with electronics recycling in China and South East Asia. Much of the recycling industry there takes place within the informal sector, and the use of personal protective gear (e.g. respirators) is poorly regulated and uncommon. Large-scale epidemics of lead poisoning were reported in China in 2009, involving more than 2,000 children living near smelting plants and sparking riots (Parry 2009; Watts 2009). The presence of total coliform bacteria in drinking water causes various water borne diseases like nausea, vomiting, diarrhoea, gastroenteritis, etc. (WHO 1980; Daniels et al. 2000; Paul 2003). The heavy metal toxicities causes nausea, persistent vomiting, diarrhoea and abdominal pain are the hallmark of most acute metal ingestions (Stenhammar 1999). Diarrhoea is found to be the common ailment in toxicity of heavy metals and coliforms.

Bholakpur is a working class locality of Hyderabad, India with a population of 34,672, wherein many small-scale tanneries are located in the area that process leather and may cause contamination of drinking water. Most of the people in the Bholakpur area do salting of animal hides for livelihood. The general hygiene of the area is very poor. Initially, five people died and later death toll rose to 16, and over 500 people were hospitalized due to diarrhoeal illness (The Hindu News Paper 2009). The present study was undertaken with the objective to assess the quality of drinking water in the Bholakpur area.

Materials and methods

The drinking water samples from municipal taps were collected from the Gulshan Nagar-I and II, Tazeer Nagar, Indira Nagar-I and II, Bangladesh market, and Mandigalli-I and II areas of the Bholakpur. These water samples were collected with the immediate effect of the diarrhoeal illness. A total of eight water samples were collected in pre-sterilized bottles (Schott Duran, Germany), and were stored at 2° to 4°C.

Physico-chemical parameters

The physico-chemical properties such as hydrogen ion concentration (pH), electrical conductivity (EC), total dissolved solids (TDS) and dissolved oxygen (DO) in water samples were analyzed on pH/EC/DO Bench top meter (Thermo Electron Corp. Orion 5 star), using standard procedures (APHA 1985).

ICP-MS analysis

The chemical analyses of the water samples were carried out using ICP-MS (Perkin-Elmer Sciex Elan DRC II). ICP-MS is a type of mass spectrometry based on coupling together inductively coupled plasma as a method of producing ions (ionizations) with a mass spectrometer as a method of separating and detecting ions. ICP-MS is highly sensitive and capable of determination of a range of metals and several non-metals at concentrations below one part in 10^{12}.

Preparation of water sample for ICP-MS analysis In the study, total 27 elements (Li, Be, B, Na, Mg, Al, Si, K, Ca, V, Cr, Mn, Fe, Ni, Co, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Sb, Ba and Pb) were determined in water samples using ICP-MS. About 100 ml of the each water sample was filtered using Whatman filter paper no.1 and collected in sample bottle and 3% nitric acid (HNO_3) was added (Rasheed et al. 2011).

Microbiological analysis

Microbiological quality of water was determined using standard plate count (SPC), membrane filtration technique, thermo tolerant coliforms (TTC) and most probable number (MPN) methods (International Organization for Standardization 2000). The tests were performed within 24 h of sample collection. The total heterotrophic bacteria (THB) was determined by SPC method using plate count agar. The plates were incubated at 37°C for 24 h and the total number of colonies was reported in colony forming units per milliliter (cfu/ml) which relates to the original sample (WHO 2004). The membrane filters provide a rapid and useful means of sampling from water. Such filters are also used for viable counting by laying on a suitable agar plate and allowing to form colonies. Acetate cellulose type membrane filter with 0.45 μ pore size was used for the detection of total viable bacteria using membrane filtration technique (APHA 1998). The MPN method was used to determine the presence of gas producing lactose fermenters and most probable number of coliforms present in 100 ml of water. The standard MPN method (nine multiple tube dilution technique) was used for detection of total coliforms by inoculation of samples into tubes of lactose broth (LB) and incubation at 37 ± 1°C for 48 h. The positive tubes were sub-cultured into Brilliant Green Lactose Broth (BGLB) and were incubated at 44.2°C for 48 h and checked for TTC. The culture media were obtained from Hi-Media Chemicals, Mumbai.

Results and discussion

The results of physico-chemical properties, ICP-MS analysis and bacteriological quality of water samples collected from the Bholakpur area are as follows:

Physico-chemical properties of water samples

The pH of water samples ranged from 7.14 to 8.70 with a mean of 7.96. According to WHO for drinking water quality, the permissible limit of pH is 6.5 to 8.0. The statistical analysis of physico-chemical properties of drinking water quality are given in Table 1. The EC of water samples ranged from 455 to 769 μS/cm with a mean of 496 μS/cm. The TDS were found to be between 303.51 to 515.23 ppm with a mean of 332.32 ppm and DO ranged between 0.28 and 6.83 mg/L with a mean of 4.03 mg/L. The permissible limit of the DO in drinking water should be ≥6 mg/L whereas the dissolved oxygen is found to be low in the samples of the Bholakpur. The source, raw water temperature, treatment and chemical or biological processes taking place in the distribution system influence the dissolved oxygen content of water. It can also cause an increase in the concentration of ferrous iron in solution, with subsequent discoloration at the tap when the water is aerated (WHO 2004).

Trace, heavy and rare earth elements analysis using ICP-MS

The concentrations of lithium (Li), beryllium (Be), boron (B), sodium (Na), magnesium (Mg), aluminium (Al), silicon (Si), potassium (K), calcium (Ca), vanadium (V), chromium (Cr),

Table 1 The statistical analysis of physico-chemical properties in water samples of Bholakpur area

Parameters	pH	EC (μS/cm)	TDS (ppm)	DO (mg/L)
No. of samples	8	8	8	8
Minimum	7.14	455	303.51	1.01
Maximum	8.72	769	515.23	6.83
Arithmetic mean	7.96	496	332.32	4.03
Median	7.95	506	339.02	1.51
Mode	7.96	506	339.02	1.52
Standard deviation	0.74	130.29	88.03	2.07
WHO permissible limits	6.5–8.0	1500	<600	≥6.0

manganese (Mn), iron (Fe), nickel (Ni), cobalt (Co), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), rubidium (Rb), strontium (Sr), molybdenum (Mo), silver (Ag), cadmium (Cd), antimony (Sb), barium (Ba) and lead (Pb) in drinking water samples are presented in Table 2.

The concentration of Fe in water samples ranges from 0.12 to 1.13 mg/L. The permissible limit of Fe concentration is 0.3 mg/L (WHO 2004); 37.5% of the samples are above the permissible limit. Acute Fe toxicity causes vomiting, gastroenteritis, haemorrhage, cardiac depression, metabolic acidosis. Fe ingestion in large quantities results in a condition known as heamochromatosis (normal regulatory mechanism do not operate effectively), where in tissue damage results from iron accumulation (WHO 1984). The concentration of Pb in water samples ranges from 0.01 to 0.07 mg/L. The permissible limit of Pb concentration in drinking water is 0.01 mg/L (WHO 2004). 37.5% of the samples are above the permissible limit and may inflict detrimental effects on the inhabitant's health. The main sources of Pb contamination of the aquatic environment are the industrial discharges from smelters, battery manufacturing units, runoff from contaminated land areas, atmospheric fallout and sewage effluents. Use of land pipes and plastic pipes stabilized with lead contribute higher levels of lead in drinking water (Pillai 1983). Pb toxicity causes nausea, vomiting, gastroenteritis, nervous system, respiratory disorders and anaemia. Children are more sensitive to Pb poisoning, which may lead to damage of brain (WHO 1972). The concentration of Cu in water samples ranges from 0.01 to 0.19 mg/L. The desirable limit of Cu in drinking water is 0.05 mg/L (ISI 1983); 12.5% of the samples show high Cu concentration. Cu is an essential nutrient, but at high doses it has been shown to cause stomach and intestinal distress, liver and kidney damage and anaemia (USEPA 1999). Acute Cu toxicity causes headache, nausea vomiting, gastrointestinal irritation, haemorrhage, haemolysis, multi-organ dysfunction syndrome (Stenhammar 1999).

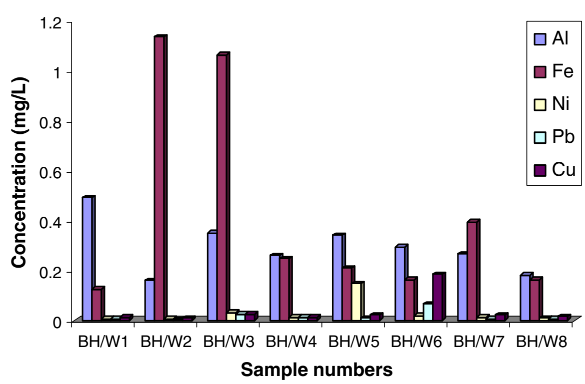
Table 2 Concentrations of trace, rare earth elements and heavy metals (mg/L) in drinking water samples of Bholakpur area

Elements	Maximum	Minimum	Average	Stdev.	WHO (2004)
Li	0	0	0	0	N.A
Be	0	0	0	0	N.A
B	0.16[a]	0.10	0.02	0.12	0.3
Na	68.69	38.36	9.72	48.60	<20
Mg	17.52	10.51	2.39	12.16	30
Al	0.49	0.16	0.11	0.29	0.2
Si	0.17	0.12	0.02	0.14	N.A
K	50.85	2.99	16.53	10.35	10
Ca	55.54	26.51	11.47	35.29	75
V	0.05	0.03	0.01	0.04	N.A
Cr	0.01	0.01	0.00	0.01	0.05
Mn	0.07	0.01	0.02	0.02	0.5
Fe	1.13	0.12	0.42	0.44	0.3
Ni	0.15	0.00	0.05	0.03	0.02
Co	0	0	0	0	N.A
Cu	0.19	0.01	0.06	0.04	2
Zn	0.41	0.11	0.10	0.20	3
As	0.01	0.01	0.00	0.01	0.01
Se	0.01	0.00	0.00	0.00	0.01
Rb	0.06	0.00	0.02	0.01	N.A
Sr	0.38	0.24	0.05	0.28	N.A.
Mo	0.04	0.00	0.01	0.01	0.07
Ag	0	0	0	0	N.A
Cd	0	0	0	0	0.003
Sb	0	0	0	0	0.005
Ba	0.13	0.05	0.03	0.07	0.7
Pb	0.07	0.00	0.02	0.02	0.01

N.A. not available, *Stdev.* Standard deviation
[a]mg/L

Records from case-study reports of gastrointestinal illness induced by copper from contaminated water or beverages plus public health department reports for 68 incidents indicate an acute onset of symptoms. Symptoms generally appear after 15–60 min of exposure; nausea and vomiting are more common than diarrhoea (Stenhammar 1999). The concentration of Ni in drinking water ranges from 0.01 to 0.15 mg/L. The desirable limit for Ni is 0.02 mg/L for drinking water (WHO 2004); 12.5% of the samples are above the desirable limit. Ni compounds induce nasal, laryngeal and lung cancer (Lessard et al. 1978). Al concentration in water samples ranges from 0.16 to 0.49 mg/L. The desirable limit of Al in drinking water is 0.2 mg/L (WHO 2004); 75% of the samples show high Al concentration. Al has been considered to be causative agent for various neurological disorders including Alzheimer's disease (Gardner and Gunn 1995). Na concentration in the water samples ranges from 38.36 to 68.69 mg/L with desirable limit of <20 mg/L (WHO 2004); 100% of the samples are above the desirable limit. Na is involved in transmission of nerve impulses and maintenance of water, acid–base balance. Water softeners increase the sodium content of drinking water.

The concentration of Zn in the drinking water ranges from 0.11 to 0.41 mg/L. The desirable limit of Zn for drinking water is specified as 3 mg/L (WHO 2004). All the water samples are within the desirable limits of WHO. Zn is an essential trace element for organisms and plays an important role in the physiological and metabolic processes of many organisms, however in high concentrations Zn can be toxic (Pillai 1983). The concentration of Cr in the drinking water found to be 0.01 mg/L, within the desirable limit of Cr specified as 0.05 mg/L for drinking water (WHO 2004). Cr is not acutely toxic to humans. Cr ($^{+}6$) is more toxic than Cr ($^{+}3$) because of its high rate of adsorption through intestinal tracts. In the natural environment, Cr ($^{+}6$) is likely to be reduced to Cr ($^{+}3$), there by reducing the toxic impact of chromium discharges. Epidemiological studies have shown a positive relationship between occupational exposure to chromates and cancer incidence. Slightly soluble hexavalent Cr salts, especially calcium chromate, are the most potent carcinogens (Moore and Ramamoorthy 1984). The concentration of Co in the drinking water was nil. The concentration of Mn in the drinking water is observed from 0.01 to 0.07 mg/L. The desirable limit is specified as 0.05 to 0.5 mg/L for drinking water (WHO 2004). All the samples show Mn concentrations within the desirable limit. Mn is a vital micronutrient and contributes to the normal development of connective tissues, besides being necessary for respiratory enzymes. It is present in high concentrations in mitochondrial fraction of human kidney, liver, and pancreas. Very large doses of infested Mn can cause some diseases and liver damages (Underwood 1977). The recommended permissible limit of Cd in drinking water is 0.003 mg/L and its concentration in the study area ranges from 0 to 0.0004 mg/L, which is within the recommended limit. Cd is a deadly poison, but a small amount taken over a long period accumulates in the biological system and causes serious illness. The major effects in the persons occupationally exposed to Cd are lung diseases and renal functions (Fleischer et al. 1974; Friberg et al. 1974). The concentrations of Fe, Pb, Cu, Ni, Al and Na exceed the permissible limits of drinking water quality, whereas none of the water samples analyzed for Li, Be, B, Mg, Si, K, Ca, V, Cr, Mn, Co, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Sb and Ba exceed the limit permitted by WHO. Heavy metal concentrations in samples of Bholakpur study area are shown in Fig. 1.



*BH/W1 to BH/W8: Bholakpur water sample identification numbers.

Fig. 1 Heavy metal concentrations in samples of Bholakpur study area

Table 3 Results of Bacteriological quality of drinking water samples of Bholakpur area

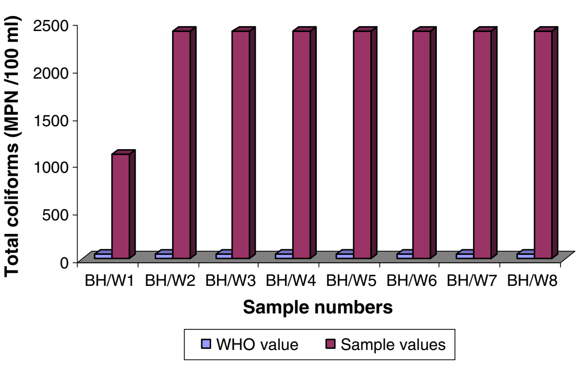
Sl.No.	Sample numbers (sample area).	Total viable bacteria (cfu/100 ml)	Total heterotrophic bacteria (cfu/100 ml)	Total coliform bacteria (MPN Index per 100 ml)
1	BH/W-1[a] (Gulshan Nagar-I)	TNC	1.0×10^5	1,100
2	BH/W-2 (Tazeer Nagar)	TNC	18×10^7	>2,400
3	BH/W-3 (Gulshan Nagar-II)	TNC	2.0×10^6	>2,400
4	BH/W-4 (Indira Nagar-I)	TNC	2.0×10^6	>2,400
5	BH/W-5 (Indira Nagar-II)	TNC	3.0×10^7	>2,400
6	BH/W-6 (Bangladesh market)	TNC	1.6×10^5	>2,400
7	BH/W-7 (Mandigalli-I)	TNC	1.0×10^6	>2,400
8	BH/W-8 (Mandigalli-II)	TNC	3.0×10^5	>2,400

TNC too numerable to count, *cfu* colony forming unit

[a]BH/W-1: Bholakpur Water sample identification number

Indicators of bacteriological quality of water

THB and total coliform bacteria are used extensively as a basis for regulating the microbial quality of drinking water. The results of the bacteriological examination of water samples collected from the Bholakpur area of Hyderabad are given in Table 3. The total heterotrophic bacteria in water samples ranged from 1.0×10^5 to 18×10^7 cfu/ml. According to WHO guidelines for drinking water quality, the total heterotrophic bacteria should be <10 cfu/ml at 37°C. In the study, all the water samples exceed the permissible limits of World Health Organization and Bureau of Indian standards (BIS). The total viable bacteria in all the water samples were found to be too numerable to count (TNC). Excessively high colony numbers of total viable bacteria indicate that the water is highly contaminated with microorganisms and is hazardous for drinking purpose, as consumption leads to various water borne diseases like nausea, vomiting, diarrhoea, gastroenteritis, etc. The most probable number is a suitable and most widely used method to determine the microbial quality of water. Present investigations have rendered the values of 1,100 to >2,400 MPN coliforms/100 ml of water which exceed the permissible limit of WHO (0 coliforms/100 ml). The total coliforms in samples of the Bholakpur study area with respect to WHO values are shown in Fig. 2. The presence of >10 coliforms/dl in water is designated as polluted or unhealthy for drinking purpose (APHA 1998). High MPN values in all the samples clearly indicate that the drinking water is highly contaminated with coliform bacteria. In this study, both THB and total coliforms are excessively high compared to the WHO and BIS guidelines. TTC test resulted in growth of coliform bacteria at a temperature of 44.2°C. Coliforms that produce acid and gas from lactose at 44.5 ± 0.2°C within 24 ± 2 h are also known as faecal coliforms due to their role as faecal indicators. The drinking water samples showing positive presumptive test, were streaked onto eosin methylene blue agar from previous positive tubes that resulted in dark centred colonies with green metallic sheen after incubation at 37°C for 24 h which shows the presence of *Escherichia coli*. *E. coli* is motile, non-spore-forming, gram-negative bacilli. Typically, they ferment lactose. Consequently, when *E. coli* is detected in the environment, it is an indication of faecal contamination (Rivera et al. 1988).



*BH/W1 to BH/W8: Bholakpur water sample identification numbers.

Fig. 2 Total coliforms in samples of the Bholakpur study area

E. coli have a central place in water microbiology for decades as an indicator of faecal pollution. It is only relatively recently that the role of *E. coli* as pathogen, rather than indicator, in drinking water has begun to be stressed. Interest in the role of *E. coli*as a cause of diarrhoeal disease has increased because of the emergence of *E. coli* O157:H7 and other enterohaemorrhagic *E. coli*, due to the severity of the related disease. There are enterotoxigenic, enteropathogenic, enterohaemorrhagic, enteroinvasive, enteroaggregative and diffusely adherent strains of *E. coli*. Each type of *E. coli* causes diarrhoeal disease through different mechanisms and each causes a different clinical presentation. Several of the types cause diarrhoea by the elaboration of one or more toxins, others by some other form of direct damage to epithelial cells (Paul 2003). A more recent outbreak affected 175 Israeli military personnel and at least 54 civilians in the Golan Heights (Huerta et al. 2000). All affected military posts and civilian communities were supplied by a common water pipeline. Samples of water from several points along the distribution system showed inadequate chlorination and high concentrations of *E. coli*. Daniels et al. in 2000 reported three outbreaks of Enterotoxigenic *E. coli* infection associated with cruise ships. All three outbreaks were associated with consuming drinks containing ice cubes on board the ship, and two were also associated with drinking unbottled water. Another outbreak affected 251 passengers and 51 crew members on a Mediterranean cruise. Enterotoxigenic *E. coli* was isolated from 13 of 22 passengers and 6 of 13 crew members sampled. Faecal coliforms were isolated from tap water, and drinking tap water was the only risk factor associated with illness in a case-control study ($p = 0.01$). There were several defects in the ship's water system including potentially faulty chlorination, and defective covers possibly allowing bilge water into the water tanks (O'Mahony et al. 1986). On a global scale diarrhoeal illness due to *E. coli* is a major cause of morbidity and mortality, especially in children. The recent emergence of Enterohaemorrhagic *E. coli* has also reawakened concern in the West about the importance of *E. coli* in food and drinking water. As already has been indicated, all enterovirulent *E. coli* are acquired directly or indirectly from a human or animal carrier. Risk from drinking water, therefore, only follows from faecal contamination of the supply (Paul 2003).

Comparative study with the control site

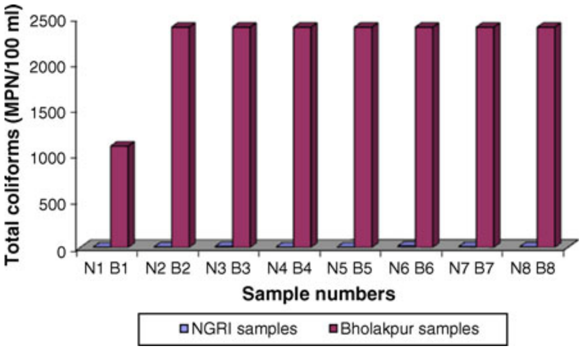
To assess the level of contamination in waters, a comparative study was performed with the control site. We have selected the National Geophysical Research Institute (NGRI) employees residential quarters of Hyderabad as a control site, where regular chlorination is done and diarrhoeal illness is not reported. The results of the bacteriological examination of water samples of the control site are given in Table 4, where the total heterotrophic bacteria ranged between 1 and 4 cfu/ml and the total coliform bacteria were found to be nil (0/MPN index per 100 ml) in all the water samples.

Total heterotrophic bacteria and total coliforms were analyzed in the water samples, as they are used extensively as a basis for regulating the microbial quality of drinking water. Comparison of total coliforms in water samples of the Bholakpur (study area) and the NGRI colony (control site) are shown in Fig. 3. All the samples of control

Table 4 Results of bacteriological quality of drinking water samples of NGRI colony, Hyderabad

Sl. no.	Sample numbers	Total heterotrophic bacteria (cfu/ml)	Total coliform bacteria (MPN Index per 100 ml)
1	NGRI/W-1[a]	2	0
2	NGRI/W-2	3	0
3	NGRI/W-3	1	0
4	NGRI/W-4	4	0
5	NGRI/W-5	2	0
6	NGRI/W-6	4	0
7	NGRI/W-7	1	0
8	NGRI/W-8	3	0

cfu colony forming unit
[a]NGRI/W-1: National Geophysical Research Institute Water sample identification number



*B1 to B8: Sample numbers of Bholakpur (B) study area.
N1 to N8: Sample numbers of NGRI (N) colony control study area where diarrheal illness is not reported for water samples.

Fig. 3 Comparison of total coliforms in water samples of the Bholakpur and NGRI colony

site are within the permissible limits of WHO and BIS, whereas all the samples in the Bholakpur area exceed the permissible limits and were found to be excessively high, which clearly states that the drinking water is contaminated with sewage. Excessively high colony numbers indicate that the water is highly contaminated with microorganisms and is hazardous for drinking purpose, as on consumption leads to various water borne diseases like diarrhoea, gastroenteritis etc. Bacteriological pollution of drinking water supplies lead to diarrhoeal outbreak in the Bholakpur area, total 16 people died and over 500 people were hospitalized due to diarrhoeal illness. Most of the people in the Bholakpur do salting of animal hides for livelihood, where the general hygiene of the area is very poor. The area has a large number of small-scale tanneries and tannery waste is highly supportable to increase the growth of microorganisms in water. The diarrhoeal illness in the Bholakpur may be due to the infiltration of contaminated water (sewage) through cross connection, leakage points and back siphonage. Hence, the quality of the water consumed is critical in controlling infectious diseases and other health problems. A regular monitoring of water quality for improvement not only prevents disease and hazards but also checks the water resources from getting further polluted (Trivedi and Goel 1986). The conservation of water sources is very important to provide safe water. As far as possible, water sources must be protected from contamination by human and animal waste, which may contain a variety of bacterial, viral, protozoan and helminth parasites. The control of drinking water quality in distribution networks remains a major challenge in urban areas. The protection of sources, treatment and distribution management are all-critical strategies in maintaining and improving piped water supplies. Thus, as far as sample waters are concerned the potential risk of getting infected by waterborne diseases is always there if used without proper disinfections. Prevention of waterborne outbreaks of diarrhoeagenic *E. coli* rests on adequate disinfection of drinking water supplies.

Toxicity of heavy metals and coliforms causing diarrhoea

In the present study area, the total coliform bacteria were extremely high in drinking water, which lead to various water borne diseases like nausea, vomiting, diarrhoea, gastroenteritis, etc. The elemental concentrations of Fe, Pb, Cu, Ni, Al and Na were found to exceed the permissible limits of WHO. The heavy metal toxicities causes nausea, persistent vomiting, diarrhoea and abdominal pain are the hallmark of most acute metal ingestions (Stenhammar 1999). Diarrhoea is found to be the common ailment in toxicity of heavy metals and coliforms. The drinking water samples were highly contaminated with high numbers of coliforms and in addition increased concentrations of Fe, Pb, Cu, Ni, Al and Na which lead to diarrhoeal outbreak in the Bholakpur locality.

Resistance of coliforms to heavy metals

In the present study, both coliforms and heavy metals were found in elevated concentrations, previous studies showed that coliforms are resistance to heavy metals. Antibiotic resistance, mediated by extrachromosomal elements or R factors, is widespread among the Enterobacteriaceae members (Watanabe 1971). *R* factors may mediate resistance to as many as eight antibiotics simultaneously and confer resistance to heavy metals such as nickel, mercury and cobalt

(Smith 1967). They are transmissible among gram-negative bacteria such as *E. coli*, *Salmonella* and *Shigella* and to other unrelated bacteria such as *Pseudomonas aeruginosa* (Datta 1975). In a sewage treatment plant of Rio de Janeiro, found that fecal coliforms in sewage waters were resistance to antibiotics and heavy metals (Ribeiro Dias et al. 1987). Ghosh et al. (2000) found that some enterobacteria isolated from nosocomial infections harboured a conjugative plasmid (>56.4 kb) encoding resistance to antibiotics and heavy metals.

Conclusions

From the results obtained it can be concluded that water samples of the Bholakpur were acceptable for most of the physico-chemical parameters, except DO. The elemental concentrations of Fe, Pb, Cu, Ni, Al and Na were found to exceed the permissible limits of WHO, whereas the bacteriological indicators were found to be excessively high, which clearly states that the drinking water is contaminated with sewage. All the samples exceed the permissible limits. Excessively high colony numbers indicate that the water is highly contaminated with microorganisms and is hazardous for drinking purpose, as on consumption leads to various water borne diseases like diarrhoea, gastroenteritis etc. Bacteriological pollution of drinking water supplies caused diarrhoeal illness in the Bholakpur, which is due to the infiltration of contaminated water (sewage) through cross connection, leakage points and back siphonage. In piped supplies, discontinuity increases the likelihood of contamination as the risk of back siphonage into the distribution network is increased when pipes are at lower pressure than the surroundings, which often contains leaked out effluent from leaking sewers. Thus, water quality indicates that pollution of the water is increasing alarmingly and that it has created serious threat to human health.

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